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UNDERSTANDING THE EFFECT OF CALCIUM-DEFICIENT DIET ON THE COMPOSITION OF GROWING BONE

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UNDERSTANDING THE EFFECT OF CALCIUM-DEFICIENT DIET ON THE COMPOSITION OF GROWING BONE

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ABSTRACT: Feeding a calcium-deficient diet to pigs and dogs leads to reduced weight gain and changes in bone structure. Detailed tables illustrate results of analysis of bones and bone ash from animals fed calcium-rich and calcium-deficient diets.

The effect of calcium deficiency, as we know, is evidence to varying degrees depending on the stage of development of the animal. While the adult organism can survive very low amounts of calcium, growing animals can suffer severe damage in the event of insufficient calcium being present for the development of the bones. /95*

The influence of a calcium-deficient diet on bone growth and bone structure has been the subject of numerous studies. These have revealed that the bones of growing animals on diets which are deficient in calcium but are otherwise adequate contain more water and less minerals than normal bones, like rachitic bones (H. Brubacher [1]). The composition of the bone ash is also modified, but only slightly, according to H. Aron [2, 3]. In the experiments which he performed on dogs together with R. Sebauer, Aron found that the calcium content of the bone ash from animals that had been raised on calcium-deficient diets had been modified only slightly with respect to normal levels.

The results of an experiment which I carried out [4] on growing pigs fed on corn (i.e., food poor in calcium but relatively rich in magnesium) showed that there is a close relationship between Ca, Mg and P_2O_5 metabolism: when corn is fed exclusively, the animals show a pronounced Ca and P_2O_5 deficiency, but large amounts of Mg are acquired. If calcium carbonate is added to the corn, there is a pronounced retention of the calcium, while the phosphorous deficiency /96

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disappears simultaneously and the storage of Mg drops to a minimum. The fact that the excreted calcium could come only from the bones and that equally large amounts of Mg were retained by the organism raise the question of where and in what form the Mg was stored. It is possible that the Mg was retained as a substitute for calcium in the bones, so that the composition of the bone ash was changed as a result.

The purpose of the experiment suggested by Professor F. Tangl, described here, was to investigate whether prolonged feeding of calcium-deficient food actually causes no change in the composition of the ashes from growing bones.

I. Experimental Organization

The experiments were carried out on growing pigs. Six young pigs from the same strain and equal in age were used, selected so that each of the two groups of three animals each had the same total weight. Three animals (1, 2, 3) received a calcium-rich diet for a long period of time, while three (4, 5, 6) received a calcium-deficient diet. The latter consisted initially of corn and gluten, later of corn and dired blood. We mixed 10 kg of corn with 1 kg of glu en or 1 kg of dried blood, added 50 g of sodium chloride to the mixture to make it appetizing, and after it had been thoroughly mixed, divided it into two halves: one had nothing added to it, while 80 g of calcium carbonate were added to the other.

The CaO and MgO content of the dry substance in the two diets was as follows: /97

	CaO	MgO
Calcium rich diet	0.448%	0.205%
Calcium deficient diet	0.055%	0.205%

The animals were fed as follows: a portion of the food was given to them three times a day, and they were allowed to eat as much as they wished. If the mixture (totalling 11 kg) was completely consumed, a new mixture was prepared. In this fashion, the animals were kept from 15 January until 7 July, 1913. Without any previous indication having been evident, Numbers 2 and 3 of the animals that had been fed the calcium rich diet suddenly died on 7 July. The autopsy revealed that the cause of death was swine fever, whereupon the remaining four animals were inoculated with serum against swine fever. For the sake of comparison, Number 5 of the animals that had been raised on a calcium deficient diet was killed by bleeding on 10 July. The experiment was continued in the manner described with the remaining animals and was terminated for the animals raised on the calcium rich diet on the 27th of September, for the animals raised on the calcium poor diet as follows: No. 4 on 30 September and No. 6 on 1 October. On these days, the animals were also killed by bleeding. During the experiment, the animals were weighed every eight days.

Processing and investigation of the bones was carried out as follows:

Initially the weight of the bones which had been carefully stripped of the muscles was determined and the bones were photographed. The fresh bones were then kept six to eight days in a large drying chamber heated to 90-95°. After they had been stored for another forty-eight hours at room temperature, they were weighed again and chopped into hazel-nut sized pieces using a strong sharp bone saw with special emphasis being placed on not losing any of the bone. The cut up bones were then placed in a two-liter flask and thoroughly boiled with ether on a reflux condenser and the dry residue (fat) in the ether solution was weighed. The bones, after being extracted and dried at 95-100°, were left in the air for forty-eight hours more and then ground to a fine meal, used for further study, especially for determining the remaining water and fat content. The bones from the four animals that were killed were processed in this manner, while the weight of the fresh bones of the animals that died of swine fever was not determined.

The chemical investigation covered the determination of the dry substance, /98
fat and ash content of the bones. In addition, the quantitative composition of the ash was determined.

The water and fat content were determined in the manner described above. The mineral content was determined by ashing the bone meal in a muffle furnace. The ash analysis included the determination of amounts of Ca, Mg, Na, K, P, S and CO_2.

To determine the Ca and Mg, the ashes were dissolved in hydrochloric acid, the solution mixed with ammonia to make it slightly alkaline, acidified with acetic acid, after which the Ca was precipitated with ammonium oxalate in the solution which had been heated to boiling, then weighed as calcium oxide. The filtrate was boiled down, mixed with ammonia and the magnesium precipitated with sodium phosphate. The ammonium magnesium phosphate was allowed to stand for twenty-four hours, eluted with water containing ammonia, dried and calcined.

For determination of the alkali, two to three grams of ashes were dissolved in the quantity of hydrochloric acid required to produce the solution, after which first ferric chloride was added to the heated solution and then ammonia until a strongly alkaline reaction was produced. In this fashion, calcium, magnesium, the added iron and phosphoric acid were removed. The voluminous precipitate was thoroughly rinsed away with hot water, the filtrate concentrated by boiling, allowed to stand and eventually filtered once more; then the clear filtrate was slightly acidified with sulphuric acid, evaporated, the existing sulphuric acid carefully driven off and the residue slightly calcined. Since the latter was often colored slightly red by traces of iron remaining in the solution, it was dissolved in hot water, the iron oxide filtered off, and evaporated, dried and carefully calcined following appropriate elution. In this fashion, the K and Ma were weighed as the sulfate and their amounts determined on the basis of the SO_4 content. Their phosphoric acid in the ash was precipitated in the nitric acid solution with ammonium molybdate, the precipitate dissolved in ammonia, again precipitated with a magnesia mixture and weighed after appropriate treatment as the magnesium pyrophosphate. The sulphuric acid was precipitated from the hydrochloric acid solution of the ashes as barium sulfate. The carbonic acid content of the ash was determined by gravimetry.

The composition of the bones could be calculated on the basis of the mineral content of the fat-free dry substance and the results of the ash analysis. The carbonic acid content of the bones, however, had to be determined separately, since it was considerably modified by calcination. To determine the carbonic acid, three to four grams of bone meal were placed in a carbonic acid determination apparatus provided with a small radiator; the carbonates was broken down with dilute sulfuric acid and the liberated carbonic acid (after complete drying) was captured and weighed in a potassium apparatus. Since the majority of the carbonic acid was retained as calcium carbonate, its amount (based on the fat-free dry substance/, could also be calculated as calcium carbonate.

3. Body Weight /99

As we already mentioned, the six experimental animals were divided into two groups so that the total weight of each group of three animals was the same at the start of this experiment on 15 January. During the first five months of the experiment, i.e., until the 15th of June, both groups received the same amount of food, i.e., 120 kg of corn and 12 kg of gluten each; one group also received 960 g of calcium carbonate. Despite the fact that they both received the same amount of food, the two groups grew in markedly different fashions. The increase in body weight was as follows from the beginning of the test until the 15th of June.

Date	With calcium	Without calc.	Date	With calcium	Without calcium
	Fed			Fed	
15 Jan.	17.70 kg	17.60 kg	2 Apr.	27.00 kg	25.40 kg
22	18.23 kg	17.35 kg	9	27.10 kg	26.18 kg
29	19.90 kg	18.82 kg	16	29.00 kg	26.85 kg
6 Feb.	20.19 kg	19.33 kg	23	30.00 kg	28.72 kg
13	20.22 kg	18.95 kg	30	31.90 kg	29.06 kg
20	21.39 kg	20.36 kg	7 May	32.95 kg	30.70 kg
27	22.07 kg	21.20 kg	14	33.50 kg	31.76 kg
8 Mar.	22.58 kg	21.81 kg	21	31.15 kg	33.01 kg
12	22.96 kg	22.85 kg	28	36.28 kg	33.95 kg
19	23.48 kg	23.20 kg	4 Jun.	39.90 kg	36.03 kg
26	23.78 kg	23.40 kg	11	39.88 kg	36.90 kg
			15	41.83 kg	36.84 kg

Hence, while the three animals that were fed the calcium-rich diet gained 24.13 kg in 151 days (= 135%), the weight increase in the animals that received the calcium-deficient diet was simultaneously 19.24 kg (= 110%).

Beginning on the 15th of June, 1 kg of blood meal was added to the 10 kg of corn instead of the 1 kg of gluten. Both groups ate their food equally well and consumed equal amounts of it until the 7th of July. The difference previously observed in weight gain now suggested to us that we should also determine individually the body weight of all these experimental animals.

The weight of the animals fed on calcium-rich diets was as follows:

Number of animal	Body weight on 15 June kg	25 June kg	2 July kg
1	17.88	17.36	19.22
2	14.65	15.23	16.30
3	9.30	9.50	9.90
	41.83	42.09	45.42

The weight of the calcium-deficient animals was as follows on 15 June, /100
25 June and 2 July:

Number of animal	Body weight on 15 June kg	25 June kg	2 July kg
	13.50	14.43	14.50
5	13.50	13.32	13.50
6	9.84	10.20	10.16
	36.84	37.95	38.16

On 7 July, Nos. 2 and 3 died from among the animals fed a calcium-rich diet; at the same time, No. 5 from among the calcium-deficient animals was killed. The three remaining animals were weighed each week from 7 July until the end of the experiment:

Date	Animal on calcium--rich diet No. 1 kg	Animal on calcium-deficient diet No. 4 kg	No. 6 kg	Together
7 July	19.6	14.50	9.90	24.40
16	20.0	15.60	10.60	26.20
23	21.3	15.80	11.10	29.90
30	23.9	15.70	11.40	27.50
6 Aug.	25.5	16.00	11.50	27.50
13	26.2	15.32	11.50	26.82
20	28.8	15.26	11.20	26.46
27	29.6	14.15	11.14	25.29
3 Sept.	35.1	13.62	11.00	24.62
10	34.2	13.00	11.80	24.80
17	34.8	12.50	11.70	24.30
24	35.7	12.60	11.12	23.72
1 Oct.	--	--	11.20	--

The continuation of the experiment therefore led to an intensification of the previously observed difference in the increase in weight of the two groups. We can see that while the body weight of the calcium-rich animals increased steadily until the end of the experiment, the calcium-deficient animals showed a stagnation and in the case of animal No. 1 even a loss of body weight.

Unfortunately we were unable to determine the amount of food eaten during the summer months, so that the weight gain and the amount of food eaten could not be compared. It is certain however that the hunger of the calcium-deficient /101 animals showed a steady decline and that they ate less food toward the end of the test than they did at the beginning.

The determination of the body weight also showed that initially the growth was only slightly affected by the lack of calcium.

However, the calcium deficiency had an effect on the growth of the animals after several weeks: the calcium-deficient animals, fed the same amount of food, grew 25% less than the calcium-rich animals. The same can be concluded from the tests performed by Aron and Sebauer [5]. Four young dogs, two of which received a calcium-deficient diet while the others received a calcium-rich diet, grew as follows during a fifty-day experiment:

Length of period	Body weight at end of the period				Change in body weight during the period			
	Calcium-rich		Calcium-poor		Calcium-rich		Calcium-poor	
	I g	II g	III g	IV g	I g	II g	III g	IV g
1. Day	2200	2000	1815	1920				
1.—10. „	2900	2670	2200	2400	+ 700	+ 670	+ 385	+ 480
11.—20. „	3300	3040	2400	2300	+ 400	+ 370	+ 200	— 100
21.—30. „	3900	3470	3100	3090	+ 600	+ 430	+ 700	+ 790
31.—40. „	4550	3450	3850	3900	+ 650	— 20	+ 750	+ 810
41.—50. „	4400	3870	4250	4620	— 150	+ 420	+ 400	+ 720
Total weight gain	2200	1870	2435	2700				

Note: Commas indicate decimal points.

During the fifty-day duration of the experiment, dog No. 1 ate 22.5 kg, dog II, 19.46 kg, dog III 22.05 kg, dog IV 20.41 kg.

The weight increase of calcium-deficient animal No. 1 was 100%, that of animal No. 2, 93.5%, while for the calcium-rich animals the figures were 134% for No. 3 and 140% for No. 4. The influence of the calcium deficient diet is initially less significant and only becomes greater if the calcium deficiency becomes too serious. This can also be seen from the experiment of Voit [6].

One of his experimental dogs, that was fed meat, bacon and distilled water, changed its body weight during an experiment lasting 162 days as follows: /102

Length of period	Body wt. at the end of the period	Amount of food eaten during the period		Change in body weight for the day
		Meat	Bacon	
	g	g	g	g
1. Day	1560	—	—	—
1.—16. "	2091	2432	608	+32
17.—32. "	2289	1643	411	+12
33.—48. "	2643	2100	475	+22
49.—64. "	2971	2100	525	+23
65.—80. "	3011	2000	500	+ 3
81.—96. "	2996	2100	525	− 1
97.—112. "	3075	1900	475	+ 6
113.—128. "	3097	1700	425	+ 2
129.—144. "	3093	1800	425	+ 0
145.—162. "	2800	1800	450	−21

As we can see, the animal grew significantly in the first half of the experiment and almost doubled its body weight in 80 days. However, a reduced appetite then set in and the body weight changed only slightly during the next 80 days.

On the basis of the experiment performed, it can therefore be stated that calcium deficiency initially affects the total growth of the animal to a slight degree; however, if the calcium deficiency becomes too serious, the effect becomes increasingly severe to the point where the calcium-deficient animals no longer grow and even lose weight.

4. Appearance, Weight, Water and Fat Content of the Bones

The appearance and the other physical characteristics of calcium-deficient and calcium-rich bones were very different. The calcium deficient bones were thinner, deformed (see Figures 1 to 3), flexible, fragile and could be cut with a knife like wood.

The weight of the fresh bones, their fat content and the amount of fat-free dry substance were as follows for one calcium-rich and three calcium-deficient animals:

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	Calcium deficient animal No. 1			Calcium deficient animal No. 4		
	Fresh bone g	Fat g	Fat-free dry substance, g	Fresh bone g	Fat g	Fat-free dry substance, g
Skull	581.9	69.0	297.2	354.7	26.8	134.6
Spine	631.2	93.4	283.9	321.3	54.9	105.8
Ribs	216.6	20.9	103.0	185.5	20.7	70.0
Front extremities	649.1	103.9	283.7	369.7	61.0	129.7
Rear Extremities	596.0	145.9	229.7	381.0	59.2	151.8
Total	2,674.8	433.1	1,197.5	1,612.2	222.6	591.9

	Calcium deficient animal No. 5			Calcium deficient animal No. 6		
	Fresh bone g	Fat g	Fat-free dry substance, g	Fresh bone g	Fat g	Fat-free dry ubstance, g
Skull	336.2	27.2	117.3	300.8	21.2	110.0
Spine	316.6	52.8	100.7	284.2	35.6	96.2
Ribs	203.8	21.3	66.0	17..9	14.5	58.6
Front extremities	349.6	45.0	115.5	297.3	43.1	106.2
Rear Extremities	297.7	56.5	105.7	265.8	51.6	94.6
Total	1,503.9	202.8	505.2	1,320.0	166.0	466.6

The body weight of the animals was as follows after bleeding, including the collected blood of calcium-rich animal No. 1: 33.42 kg, calcium deficient animal No. 4: 11.24 kg, No. 5: 13.42 kg; No. 6: 10.82 kg. Since the varying content of the digestive tracts led to error in comparison of body weight, the entire intact digestive tract was removed and weighed. After its weight was calcium-rich animal No. 1 was found to weigh 30.58 kg, calcium-poor animal No. 4: 9.66 kg, No. 5: 11.90 kg and No. 6: 9.74 kg.

On the basis of these and the above data, one can calculate what percentage of the weight of the body minus the digestive tract was divided between the fresh bones and the fat-free dry substance. These data are as follows:

	Fresh bone %		Dry substance %	
Calcium-rich animal No. 1	8.74		3.91	
Calcium-poor animal No. 4	16.68	Average: 14.29%	6.12	Average: 5.05%
Calcium-poor animal No. 5	12.63		4.24	
Calcium-poor animal No. 6	13.55		4.97	

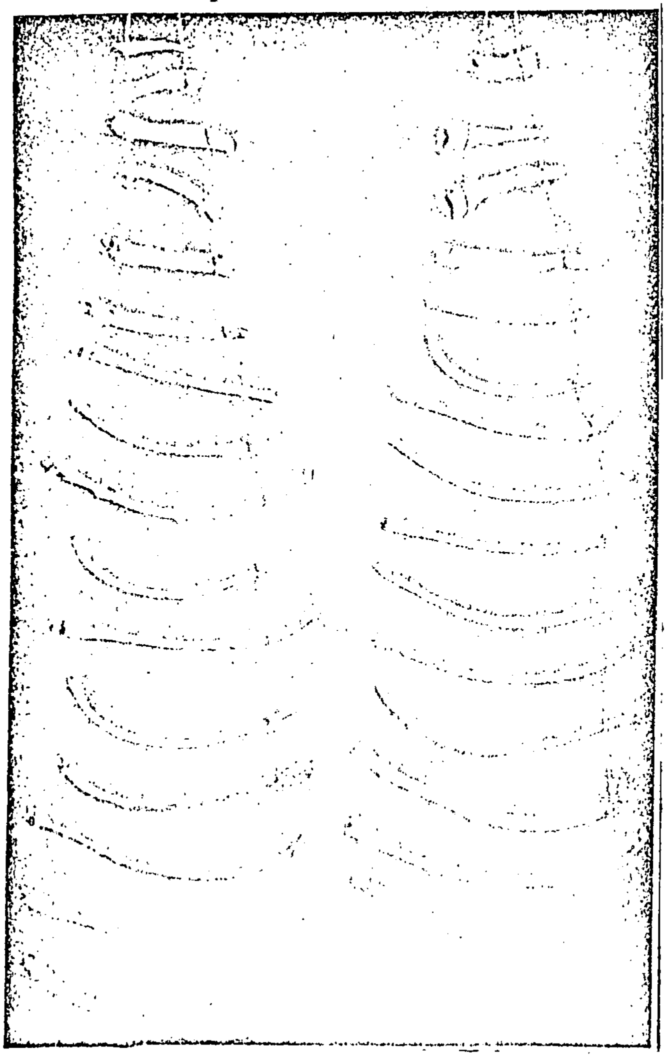


Figure 1. Ribs of Calcium-Rich Animal Number 1.

The weight of the bones, in /104
both the fresh and dry state, constituted a larger proportion of the body weight for the calcium-deficient animals than in the case of the calcium-rich animal.

The water content of the individual bones and the entire skeleton was as follows:

	Calcium rich animal	Calcium deficient animal		
	Nr. 1	Nr. 4	Nr. 5	Nr. 6
	%	%	%	%
Skull	42,06	48,95	62,04	60,31
Spine	47,22	60,29	61,83	61,32
Ribs	47,37	57,53	63,84	62,77
Front extremities . . .	47,97	58,00	62,08	58,22
Rear extremities . . .	48,97	52,83	56,18	55.84
Entire skeleton	46,59	57,41	61,16	59,57

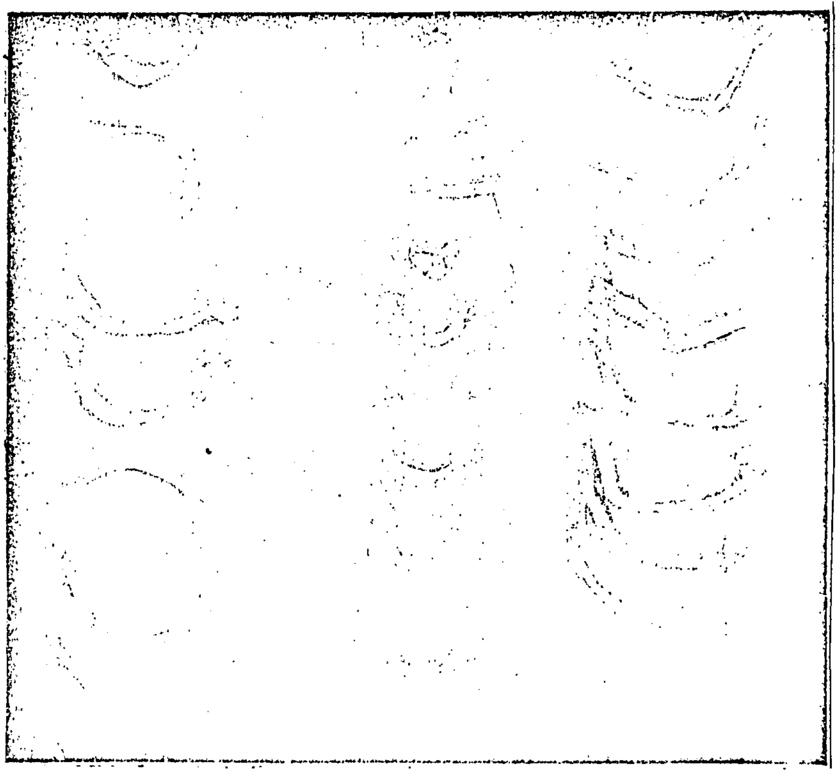
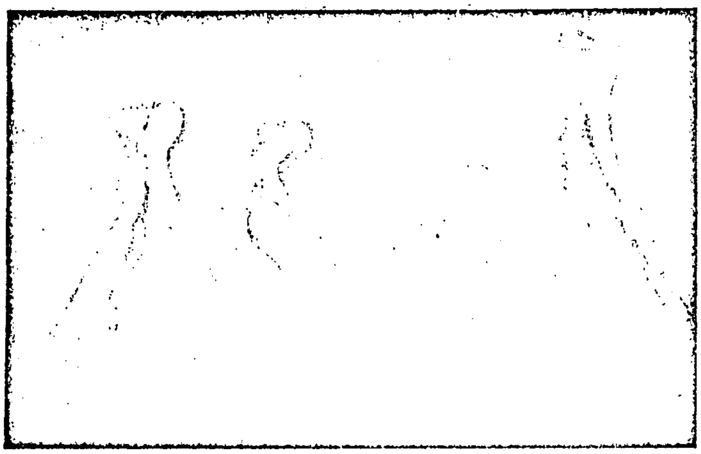


Figure 2. Ribs of Calcium-Deficient Animal Number 5.

Under the influence of the calcium-deficient diet, bones that contain more water developed. The same was found for young dogs by Aron and Sebauer [5].



a b b a

a: Shoulder blade of calcium-rich animal No. 1 /106
b: Shoulder blade of calcium-rich animal No. 5.

The water content of the bones in their experiments was as follows:

	Calcium-deficient Dog	Calcium-rich Dog
	%	%
Femur	62,77	44,16
Tibia	61,55	41,89
Fibula	62,50	45,79
Humerus	64,78	44,79
Ulna	61,99	42,19
Radius	62,50	41,04
Scapula	73,48	59,20
Atlas	58,26	39,98
Epistropheus	60,90	42,57

Note: Commas indicate decimal points.

It was also interesting to calculate the fat content of the two kinds of bones. This value was as follows for the dry substance of the individual bones and the skeleton as a whole:

	Calcium-rich animals	Calcium deficient animals		
	No. 1	No. 4	No. 5	No. 6
	%	%	%	%
Skull	18.84	16.60	18.70	16.03
Spine	24.75	34.16	34.40	27.01
Ribs	16.86	22.85	24.39	19.83
Front extremities	26.81	31.98	28.03	28.86
Rear extremities	38.84	28.06	34.83	35.29
Entire skeleton	26.56	27.32	28.64	26.27

Note: Commas indicate decimal points.

The calcium-rich animal was the one on which the experiment was performed /107 for the longest time and whose body weight rose from 6 kg to 34.77 kg. Despite the significant difference in the increase in body weight, we can see that the bones of the calcium-deficient animal were as rich in fat as those of the calcium-rich animals.

5. Ash Content and Composition of Ashes

Ash Content of Bones

	Calcium rich animals				Calcium deficient animals			
	No. 1 %	No. 2 %	No. 3 %	Avg. %	No. 1 %	No. 2 %	No. 3. %	Avg. %
Skull	60.02	60.35	58.19	*59.62*	56.83	47.02	55.50	*58.11*
Spine	40.65	39.88	41.03	*40.52*	35.22	25.18	31.18	*30.52*
Ribs	43.94	50.07	45.97	*46.66*	28.08	21.57	27.73	*25.80*
Front extremities	46.95	47.13	46.97	*47.01*	37.44	27.23	36.12	*33.60*
Rear extremities	46.76	47.36	44.68	*46.26*	28.25	30.27	40.52	*33.00*

The relationship calculated from the averages between the inorganic and organic substance (inorganic substance: organic substance) was as follows for the calcium-deficient and calcium-rich bones:

	Calcium-rich animals	Calcium-deficient animals
Skull	1.476	1.387
Spine	0.681	0.439
Ribs	0.875	0.347
Front extremities	0.887	0.506
Rear extremities	0.861	0.490

These numbers indicate that in the case of calcium deficiency the skull bones contribute relatively the least amount to the ashes while the spine and ribs contribute the most.

The percentile amount of ash for the entire skeleton can be calculated from amount of fat-free dry substance and mineral content. The mineral content of the fat-free dry substance of the entire skeleton was as follows:

for calcium rich animal No. 1	48.40%
for calcium deficient animal No. 4	40.03%
for calcium deficient animal No. 5	31.31%
for calcium deficient animal No. 6	39.56%

The difference in ash content of the entire skeleton is not so significant /108 as in the case of the individual bones. This is because most of the mineral content of the skeleton is contained in the skull bones and the difference in ash content was least for this in both types of animals.

The ash content of the skeleton was distributed as follows among the different bones:

	Calcium-rich animal	Calcium-deficient animals		
	No. 1, %	No. 4, %	No. 5, %	No. 6, %
Skull	30.77	37.39	34.86	33.42
Spine	19.91	15.73	16.03	16.25
Ribs	7.81	8.29	9.00	8.80
Front extremities	22.98	20.49	19.88	20.77
Rear extremities	18.53	18.10	20.23	20.76
Total	100.00	100.00	100.00	100.00

The composition ofthe ashes was determined in the fashion described under the section dealing with the arrangement of the experiment. Comparison of the CaO, MgO, P_2O_5, SO_3 and CO_2 content of the two kinds of bones showed a marked difference, while the sum of the above components in the case of the calcium--rich bone amounted to approximately 100%; their sum for the calcium-deficient / bones was markedly less.

A further study of the bone ashes revealed considerably amounts of alkali in the calcium deficient bones. In two to three cases I looked for similar findings in the ashes of the calcium-rich animals, but only found amounts that were so small that their determination could be disregarded, especially in view of the fact that the sums of CaO, MgO, P_2O_5, SO_3 and CO_2 amounted to more or less than 100% within the analytical limits of error.

The composition of the ashes from the two kinds of bones is listed in Table 1. For the sake of simplicity, the average values for the ashes of the individual bones are listed once again at the end.

TABLE 1. COMPOSITION OF BONE ASHES /109

Calcium-rich animals						
	CaO	MgO	P_2O_5	SO_3	CO_2	Tot.
Skull						
1.	55,21	1,13	41,72	0,78	1,90	100,74
2.	54,78	1,01	42,32	0,56	1,43	100,10
3.	53,95	1,39	43,01	0,70	0,78	99,83
Avg.	**54,64**	**1,18**	**42,35**	**0,68**	**1,37**	**100,22**
Ribs						
1.	54,05	1,00	39,85	1,40	1,80	98,10
2.	55,39	0,48	41,58	0,75	1,83	100.03
3.	55,76	1,18	42,22	0.93	0,51	100,60
Avg.	**55,07**	**0,89**	**41,22**	**1,03**	**1,38**	**99,59**
Spine						
1.	54,15	1,03	40,77	1,30	2,38	99,63
2.	54,27	0,59	42.41	1,83	0,76	99,86
3.	53.90	1,64	42,92	1,71	1,35	101,72
Avg.	**54,11**	**1,09**	**42,03**	**1,62**	**1,49**	**100,34**
Front extremities						
1.	53,69	1,26	40,19	1,17	1,78	98,10
2.	54,41	1,22	41,58	1,86	1,47	100,54
3.	55,29	1,08	41,36	1,25	0,85	99,83
Avg.	**54,46**	**1,19**	**41,04**	**1,42**	**1,36**	**99,47**
Rear extremities						
1.	54,51	0,96	40,23	1,07	1,31	98,08
2.	55,21	1,43	40,79	2,29	1,66	101,38
3.	54,35	1,57	41,55	1,01	0,79	99.27
Avg.	**54,69**	**1,32**	**40,86**	**1,45**	**1,25**	**99,57**

Calcium-deficient animals								
	CaO	MgO	Na_2O	K_2O	P_2O_5	SO_3	CO_2	Tot.
Skull								
4.	52,81	1,93	2,51	0,45	41,64	0,61	0,20	100,15
5.	51,18	1,84	4,22	0,18	40,37	0,37	0,62	98,78
6.	52,42	2.20	2,64	0,47	41,68	0,58	0,52	100.51
Avg.	**52,14**	**1,99**	**3,12**	**0,37**	**41,23**	**0,52**	**0,45**	**99,82**
Ribs								
4.	49,42	0,61	6,26	1,08	38,66	2,01	1,16	99,20
5.	47,52	1,55	6,69	1,53	39.75	1,53	0,68	99,25
6.	49,28	1,62	4,07	0,58	38,88	2.50	1.33	98,26
Avg.	**48,74**	**1,26**	**5,66**	**1,06**	**39,10**	**2,01**	**1,06**	**98,89**
Spine								
4.	50,21	1,30	5,32	0,38	39,52	2,25	1,12	100.10
5.	47,53	1,36	5,73	2,14	38,95	1,47	1,14	98,32
6.	49,95	2,29	3,64	1,29	40.52	0,98	1.66	100,33
Avg.	**49,23**	**1,65**	**4,90**	**1,27**	**39,66**	**1,56**	**1,31**	**99,58**
Front extremities								
4.	50,41	0,39	3,55	1,11	38,57	0,95	4,72	99,70
5.	49,71	1,40	5,11	1,33	38,69	1,01	1,58	98,83
6.	50,94	1,39	4,00	0,70	38,98	1.26	—	97,27[1]
Avg.	**50,35**	**1,06**	**4,22**	**1,05**	**38,75**	**1,07**	**3.15**	**99,25**
Rear extremities								
4.	50,50	1,02	4,69	0,40	38.38	1,30	4.35	100,64
5.	50,80	1,20	3,86	0,50	38,86	1,44	0,87	98,53
6.	52,92	1,56	3,96	0,17	38,66	0,96	—	98,23[1]
Avg.	**51,40**	**1,26**	**4,17**	**0,35**	**38,97**	**1,23**	**2,61**	**99,99**

1) Without CO_2

Note: Commas indicate decimal points.

	Skull bones Calcium-		Ribs Calcium-		Spine Calcium-	
	rich	poor	rich	poor	rich	poor
	%	%	%	%	%	%
CaO	54,64	52,14	55,07	48,74	54,11	49,23
MgO	1,18	1,99	0,89	1,26	1,09	1,65
Na_2O	—	3,12	—	5,66	—	4,90
K_2O	—	0,37	—	1,06	—	1,27
P_2O_5	42,35	41,23	41,22	39,10	42,03	39,66
SO_3	0,68	0,52	1,03	2,01	1,62	1,56
CO_2	1,37	0,45	1,38	1,06	1,49	1,31
Total	100,22	98,82	99,59	98,89	100,34	99,58

Note: Commas indicate decimal points.

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	Front extremities Calcium-		Rear extremities Calcium-	
	rich	poor	rich	poor
	%	%	%	%
CaO	54,46	50,35	54,69	51,40
MgO	1,19	1,06	1,32	1,26
Na_2O	—	4,22	—	4,17
K_2O	—	1,05	—	0,35
P_2O_5	41,04	38,75	40,86	38,97
SO_3	1,42	1,07	1,45	1,23
CO_2	1,36	3,15	1,25	2,61
Total	99,47	99,25	99,57	99,99

Note: Commas indicate decimal points.

The two kinds of bone ashes therefore show different compositions, with those from the calcium-deficient animals showing significant amounts of alkali. The amount of $Na_2O + K_2O$ was as follows:

The ashes of the skull	3.49%
the ashes of the ribs	6.72%
the ashes of the spine	6.17%
the ashes of the front extremities	5.27%
the ashes of the rear extremities	4.52%

The bone ashes of the calcium-deficient animals show the same composition as the ashes of rachitic children's bones.

Burbacher [1] determined the CaO, MgO and P_2O_5 content of an eight-month old rachitic child and a seven month old normal child. The totals of these components were as follows for the two types of ashes:

	Rachitic child %	Normal child %	Less in the rachitic bones %
Head bone	89.99	92.36	-2.37
Ribs and spine	84.94	87.57	-2.63
Upper extremities	84.66	89.59	-4.93
Lower extremities	77.72	87.37	-9.65
Entire skeleton	85.81	89.74	-3.93

In the experiments of Aron and Sebauer [5] the calcium content of the bones of dogs that had received a calcium-deficient diet was reduced. The values were as follows: /111

	CaO in the ashes	
	Ashes of the fibula	Mixed ashes
Calcium-deficient	50.40%	49.78%
Calcium-rich	53.40%	52.29%

Likewise, Brubacher [1] found less CaO in the bone ashes of rachitic children:

	Normal bone %	Rachitic bone %	Less in the rachitic bone %
Head bone	50,16	47,72	— 2,44
Ribs and Spine	46,15	43,86	— 2,29
Upper extremities	47,57	43,45	— 4,12
Lower extremities	46,71	39,55	— 7,16
Entire skeleton	48,09	44,72	— 3,37

Note: Commas indicate decimal points.

In my experiments also, the bone ashes of the calcium-deficient animals always contained less CaO than the ones from calcium-rich animals. The differences were as follows on the average:

for the skull bones	2.50%
for the spine	4.88%
for the ribs	6.83%
for the front extremities	4.11%
for the rear extremities	3.29%

The difference is much less in the case of phosphoric acid, as was also found by Brubacher [1] in rachitic bones.

The minimum content of P_2O_5 in my experiments occurred in the calcium-deficient animals for the ashes of the following bones:

the skull bones	1.22%
the spine	2.37%
ribs	2.12%
front extremities	2.29%
rear extremities	1.89%

There is also a difference in the MgO contents, but I was unable to attribute any particular significance to it:

	MgO in bone ashes		
	Of calcium-rich animals %	Of calcium-deficient animals %	Difference %
Skull	1.18	1.99	+0.81
Ribs	0.89	1.26	+0.37
Spine	1.09	1.65	+0.56
Front extremities	1.19	1.06	-0.13
Rear extremities	1.32	1.26	-0.06

While the ashes of the skull, ribs and spine of the calcium-deficient animals contain somewhat more MgO than the ashes of the same bones in the calcium-rich animals, the MgO content in the ashes of the forward and rear extremities are the same within the analytical limits of error.

Hence, when corn is fed exclusively, according to my previous expeyiments carried out on growing pigs [7], the Mg which is retained is not stored in the bones in place of the Ca which is excreted; only a small portion of the Mg which is retained is stored in the bones.

TABLE II. COMPOSITION OF FAT-FREE BONE DRY SUBSTANCES.

Calcium-rich animals						Calcium-deficient animals							
	CaO	MgO	P_2O_5	SO_3	CO_2		CaO	MgO	Na_2O	K_2O	P_2O_5	SO_3	CO_2
	Skull						Skull						
1.	33,14	0,67	25,04	0,47	4,32	4.	30,01	1,10	1,42	0,26	23,66	0,35	2,90
2.	33,06	0,61	26,14	0,34	4,12	5.	24,06	0,86	1,98	0,08	18,98	0,17	1,56
3.	31,39	0,81	25,03	0,41	4,11	6.	29,09	1,22	1,46	0,26	23,13	0,32	3,13
Avg.	**32,53**	**0,70**	**25,40**	**0,40**	**4,18**	Avg.	**27,72**	**1,06**	**1,62**	**0,20**	**21,92**	**0,28**	**2,53**
	Rib						Rib						
1.	23,75	0,44	17,51	0,61	5,21	4.	13,88	0,17	1,76	0,30	10,86	0,56	1,82
2.	27,73	0,24	20,82	0,37	4,55	5.	10,26	0,33	1,44	0,33	8,58	0,33	2,41
3.	25,63	0,54	19,41	0,43	6,69	6.	13,66	0,45	1,13	0,16	10,78	0,69	3,40
Avg.	**25,70**	**0,40**	**19,24**	**0,47**	**5,48**	Avg.	**12,60**	**0,32**	**1,44**	**0,26**	**10,07**	**0,53**	**2,54**
	Spine						Spine						
1.	22,01	0,42	16,57	0,53	3,67	4.	17,68	0,46	1,87	0,10	13,92	0,79	1,81
2.	21,64	0,24	16,91	0,73	4,13	5.	11,97	0,34	1,44	0,54	9,81	0,37	1,24
3.	22.11	0,67	17,61	0,70	4.14	6.	15,57	0,71	1,13	0,40	12,63	0,30	1,56
Avg.	**21,92**	**0,44**	**17,03**	**0,65**	**3,98**	Avg.	**15,07**	**0,50**	**1,48**	**0,32**	**12,12**	**0,49**	**1,54**
	Front extremities						Front extremities						
1.	25,21	0,59	18.87	0,55	5,82	4.	18,87	0,15	1,33	0,42	14,44	0,36	2,31
2.	25,64	0,57	19,60	0,88	4,24	5.	13,54	0,38	1,39	0,36	10,54	0,28	3,23
3.	25,97	0,51	19,43	0,59	4,00	6.	18,40	0,50	1,44	0,25	14,08	0,46	2,98
Avg.	**25,61**	**0,56**	**19,30**	**0,67**	**4,69**	Avg.	**16,94**	**0,34**	**1,39**	**0,34**	**13,02**	**0,36**	**2,84**
	Rear extremities						Rear extremities						
1.	25,49	0,45	18,81	0,50	5,43	4.	14,25	0,29	1,32	0,11	10,83	0,37	2,22
2.	26,14	0,68	19,32	1,08	4,25	5.	15,38	0,36	1,17	0,15	12,06	0,44	2,95
3.	24.28	0,70	18,56	0,45	2,90	6.	21,44	0,63	1,60	0,07	15,67	0,39	3,54
Avg.	**25,30**	**0,61**	**18,90**	**0,68**	**4,19**	Avg.	**17,02**	**0,43**	**1,36**	**0,11**	**12,85**	**0,40**	**2,90**

6. Composition of the Bones

Finally, we calculated the ratio of the ash residue to the bone dry substance. Giving consideration to the changing amounts of water and fat, the values were based on the fat-free bone dry substance. The data in this connection are shown in Tables II. (Carbonic acid content was determined not in the ashes but as we have already mentioned, directly in the dried and powdered bone.

The composition of the fat-free dry substance of the bone reflects the influence of the calcium deficient diet in the same fashion as in the composition of the ashes.

The CO_2 and CaO content are of particular interest. The average values for all the CO_2 determinations were as follows:

	For the calcium--rich bones		For the calcium--deficient bones	
	CO_2 %	CaO %	CO_2 %	CaO %
Skull	4.18	9.51	2.53	5.75
Ribs	5.48	12.46	2.54	5.78
Spine	3.98	9.05	1.54	3.50
Front extremities	4.69	10.67	2.84	6.46
Rear extremities	4.00	9.10	2.90	6.60

As we could expect from the results, the bone that developed under the influence of a calcium-deficient diet not only contained less calcium phosphate but by comparison much less calcium carbonate than normal bones.

If we examine these experiments, their results can be summarized as follows:

The influence of a prolonged feeding with calcium-deficient food affects the growth and body weight in such a manner that the animals even at the start grow approximately 20% less than control animals fed with a calcium-rich diet. If the calcium deficiency is significant during a long period of feeding during the experiment, the appetite of the animals decreases and their weight gain drops off markedly; the growth of the animals can not only stop but their body weight can even drop off.

The bones of the calcium-deficient animals are very different from those of the calcium-rich bones in both appearance and general physical characteristics. They are thinner, deformed, flexible, fragile and can be cut readily with a knife. /114

The growth and weight of the skeleton is the same for animals fed on a calcium-deficient diet as for those fed a calcium-rich one. The body weight of the calcium-deficient animals was made up to a larger percentage by the weight of the fresh bones and the dry substance (14.29 and 5.05%) than in the calcium-rich animals (8.74 and 3.91%). While the calcium-deficient bones were much richer in water than those of the calcium-rich animals, their fat content was almost exactly the same.

The ash content of the calcium-deficient bones was much less than that of the calcium-rich ones. The difference in the skull bones was slightest and that in the ribs was greatest. In the calcium-deficient animals, the total ash content of the skeleton was made up to a larger extent by material from the skull than in the calcium-rich animals.

The bone ashes of the calcium-deficient animals were much poorer in CaO than the ashes of the calcium-rich bone. There was a slighter difference showing the same trend in the P_2O_5 content.

The most important difference between the composition of the bone ashes of calcium-deficient and calcium-rich animals consists in the fact that the former contain significant amounts of alkalis, much more Na_2O than K_2O.

The changes in the ashes are not the same in all bones: the least amount occurs in the skull bones and the greatest amount in the ribs and spine.

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